

Attorney Docket No.: **P-756 (TI-0022)**  
Inventors: **Huber et al.**  
Serial No.: **09/770,410**  
Filing Date: **January 25, 2001**  
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This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

Claims 1-50 (canceled).

Claim 51 (original): A device for separating a mixture of polynucleotides, said device comprising:

a polymeric monolith having non-polar chromatographic surfaces,

wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,

wherein said monolith is contained within a fused silica tube having an inner diameter in the range of 1 micrometer to 1000 micrometer, wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.

Claim 52 (original): A device of claim 51 wherein said tube is devoid of retaining frits.

Claim 53 (original): A device of claim 51 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.

Claim 54 (original): A device of claim 53 wherein said theoretical plates per meter is determined from the retention time

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of single stranded p(dT)<sub>18</sub> standard using the following equation:

$$(N / L) = (5.54 / L) \left( \frac{t_R}{w_{0.5}} \right)^2$$

wherein  $N$  is the number of theoretical plates,  $t_R$  is the retention time of said standard determined during an isocratic elution,  $w_{0.5}$  is the peak width at half height, and  $L$  is the length of the monolith in meters.

Claim 55 (original): A device of claim 54 wherein said tube has an inner diameter of 200 micrometer and a length of 60mm, wherein during said isocratic elution said monolith has a back pressure in the range of 180 to 200 bar, and a flow rate in the range of 2 to 3  $\mu$ L/ min at an elution temperature of 50°C.

Claim 56 (original): A device of claim 51 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

Claim 57 (original): A device of claim 51 wherein the chromatographic surfaces of said monolith are devoid of micropores.

Claim 58 (original): A device of claim 57 wherein said monolith has channels sufficiently large for convective flow of said mobile phase.

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Claim 59 (original): A device for separating a mixture of polynucleotides, said device comprising:

a polymeric monolith having non-polar chromatographic surfaces,

wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,

wherein said monolith is contained within a fused silica tube, and

wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.

Claim 60 (original): A device of claim 59 wherein said tube has an inner diameter in the range of 1 micrometer to 1000 micrometer.

Claim 61 (original): A device of claim 59 wherein said tube is devoid of retaining frits.

Claim 62 (original): A device of claim 59 wherein said monolith is characterized by having 10,000 to 200,000 theoretical plates per meter.

Claim 63 (previously presented): A device of claim 59 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that is brush-like.

Claim 64 (original): A device of claim 59 wherein said monolith comprises an underivatized monolithic stationary phase.

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Claim 65 (original): A device of claim 59 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

Claim 66 (original): A device of claim 59 wherein said monolith is devoid of micropores and wherein said monolith has channels sufficiently large for convective flow of said mobile phase.

Claim 67 (original): A device for separating a mixture of polynucleotides, said device comprising:

a polymeric monolith having non-polar chromatographic surfaces,

wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,

wherein said monolith is contained within a fused silica tube,  
wherein said tube has been silanized, and  
wherein said tube is devoid of retaining frits.

Claim 68 (original): A device of claim 67 wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.

Claim 69 (original): A device of claim 67 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.

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Claim 70 (original): A device of claim 67 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is brush-like.

Claim 71 (original): A device of claim 67 wherein said tube has an inner diameter in the range of 1 micrometer to 1000 micrometer.

Claim 72 (original): A device of claim 67 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

Claim 73 (previously presented): A device for separating a mixture of polynucleotides, said device comprising:

a polymeric monolith having non-polar chromatographic surfaces,

wherein said monolith comprises an underivatized polystyrene divinylbenzene matrix,

wherein said monolith is contained within a tube having an inner diameter in the range of 1 micrometer to 1000 micrometer,

wherein said monolith is characterized by having 10,000 to 200,000 theoretical plates per meter.

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Claim 74 (previously presented): A device of claim 73 wherein said monolith is contained within a tube having an inner diameter in the range of 10 micrometer to 500 micrometer.

Claim 75 (original): A device of claim 73 wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.

Claim 76 (original): A device of claim 75 wherein said tube is devoid of retaining frits.

Claims 77-78 (canceled).

Claim 79 (original): A device for separating a mixture of polynucleotides, said device comprising:

a polymeric monolith having non-polar chromatographic surfaces,

wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,

wherein said monolith is characterized by having at least 100,000 theoretical plates per meter,

wherein said monolith is contained within a silanized fused silica tube having an inner diameter in the range of 10 micrometer to 1000 micrometer,

wherein said monolith is immobilized at the inner wall of said tube.

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Claim 80 (original): A device of claim 79 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.

Claim 81 (original): A device of claim 79 wherein said monolith is contained within a tube having an inner diameter in the range of 1 micrometer to 1000 micrometer.

Claims 82-83 (canceled).

Claim 84 (original): A device of claim 79 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

Claim 85 (original): A miniaturized chromatographic system for separating a mixture of polynucleotides said system comprising the device of claim 79.

Claim 86 (original): A device for separating a mixture of polynucleotides, said device comprising:

a polymeric monolith having non-polar chromatographic surfaces,

wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose and brush-like,

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wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,

wherein said monolith is contained within a fused silica tube having an inner diameter in the range of 1 micrometer to 1000 micrometer,

wherein said monolith is immobilized at the inner wall of said tube.

Claim 87 (original): A device of claim 86 wherein said tube is devoid of retaining frits.

Claim 88 (original): A device of claim 86 wherein said monolith characterized by having 100,000 to 200,000 theoretical plates per meter.

Claim 89 (original): A device of claim 86 wherein said tube has been silanized.

Claim 90 (original): A device of claim 86 wherein said surfaces of said monolith are non-porous.

Claim 91 (original): A device of claim 86 wherein said monolith is formed from a polymerization mixture including underivatized styrene, a crosslinking agent, and a porogen, wherein said porogen comprises tetrahydrofuran.

Claim 92 (original): A device of claim 86 wherein said polynucleotides comprise double-stranded fragments having lengths in the range of 3 to 600 base pairs.

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Claims 93-94 (canceled).

Claim 95 (original): A system of claim 85 wherein said monolith is operatively coupled to a mass spectrometer.

Claim 96 (canceled.)

Claim 97 (original): A chromatographic device, said device comprising:

a polymeric monolith having non-polar chromatographic surfaces,

wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,

wherein said monolith is contained within a silanized fused silica tube having an inner diameter in the range of 10 micrometer to 1000 micrometer, and wherein said monolith is immobilized at the inner wall of said tube.